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2006.03.22

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Marianne W.

Etternavn:
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SØKNAD s. 2 av 2

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Tittel:

Optical imaging of endometriosis

Optisk avbilding av endometrose

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Oslo, 29. september 2003

Signatur:
Marianne W. Wulff

Navn i blokkbokstaver:
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Optical imaging of endometriosis

Field of the invention

The present invention provides contrast agents for optical imaging of endometriosis in patients. The contrast agents may be used in diagnosis of endometriosis, for follow up of progress in disease development, and for follow up of treatment of endometriosis.

The present invention also provides new methods of optical imaging of endometriosis in patients, for diagnosis, and for follow up of disease development and treatment of endometriosis.

Description of related art

Endometriosis is a disorder that may be painful. The disorder is characterized by endometrial tissue being present in other organs than the endometrium. Typical organs where endometrial tissue may be present in endometriosis include, in addition to endometrium, the abdominal wall and the outer surfaces of organs in the abdomen including lower bowel, ovaries and fallopian tubes. Endometriosis is a disease that affects several percent of the fertile female population. Some patients are asymptomatic whereas others have symptoms such as chronic pain, dysmenhorrhoea and menstrual disturbances. Even mild endometriosis may result in infertility or reduced fertility.

The aims of treatment of a patient with endometriosis often include relief of pain and elimination of endometrial tissue outside the endometrium. Current treatments of endometriosis include surgery and/or drug treatment. Drugs used in endometriosis are drugs that suppress the activity of the ovaries and slow down the growth of endometrial cells.

Methods for diagnosis of endometriosis include *in vitro* methods using immunoassays, for instance CA-125 and endometrial antibodies, laparoscopic examinations and various medical imaging techniques such as computer tomography, ultrasound and MRI. *In vitro* assays are not sufficiently sensitive for diagnosis of endometriosis. In general, medical literature reports varying degrees of success in using medical imaging for diagnosing endometriosis. The best imaging method today is probably MRI. However, the MR signal from endometriotic tissue varies a lot and it is not possible to distinguish endometriosis from other diseases in the same organ or organ system.

Various radiolabelled monoclonal antibodies or fragments thereof have been evaluated for diagnosis of endometriosis, but again these agents show low sensitivity and/or low specificity.

The most promising diagnostic tool today for diagnosis of endometriosis is laparoscopy. However, this method has its limitation in early diagnosis of endometriosis and for follow up therapy of endometriosis.

WO 95/13821 claims a method for diagnosis of endometriosis based on assaying for ß3 integrin in an endometrium sample using a monoclonal antibody specific for ß3 integrins. There is no description of its use *in vivo*.

US 6,387,629 claims that cathepsin S expression is upregulated during endometriosis. The patent document focuses on *in vitro* assays to determine cathepsin S, however, there are claims related to *in vivo* use and specific claims on radioisotopes and paramagnetic compounds. There is no description of optical imaging methods *in vivo*. The targeting vector has a polynucleotide of at least 7 to about 50 nucleotides in length.

WO 99/63116 relates to the use of prothymosin in the diagnosis and treatment of endometriosis. The invention is based on the observation that prothymosin expression is upregulated in endometriosis. The application focuses on diagnosis of endometriosis based on prothymosin in tissue samples such as endometrial tissue, blood and urine, however, there are claims related to *in vivo* use and specific claims related to radioisotopes and MRI.

US 5,328,826 relates to immunochemical detection of human uterine endometrial cancer cells and not endometriosis *per se*.

WO 93/20810 relates to compositions comprising a precursor of PpIX for detecting and treating malignant and non-malignant tissue including diseases in endometrium. There is no description of specific use for diagnosing endometriosis.

WO 00/59547 and US 6,540,980 relates to kits for detecting or treating endometriosis comprising an eosinophil peroxidase-binding component. The targeting agent might according to the claims be labeled with a fluorescent label and might be used *in vivo*. The targeting agents are antibodies and fragments thereof. There is no disclosure of low molecular weight compounds.

WO 00/47739 relates to polypeptide antigens for detection of auto-antibodies in endometriosis patients. The diagnostic use of these polypeptide antigens includes any *in vitro* assay and does not disclose *in vivo* use.

WO 00/25789 discloses a method for preventing and treating endometriosis and other related diseases and is comprised of administering to a patient a phenyl-protein transferase inhibitor. The invention is not related to diagnosis or to low molecular weight compounds.

US 5,618,680 discloses a method for diagnosing endometriosis based on ligands which specifically binds a Major Histocompatibility Complex (MHC)-Class I antigen. The document does not discuss *In vivo* imaging.

US 5,380,317 relates to a medical device applying localised high intensity light and heat for destruction of the endometrium. The document does not relate to contrast agents.

WO 97/06797 relates to endometrial ablation using photodynamic therapy.

Although there are several patents and scientific publications on optical imaging using contrast agents with fluorescent properties in the ultraviolet to near infrared part of the spectrum, there are no documents focusing on in vivo diagnosis of endometriosis by optical imaging. As pointed out there is still a challenge to diagnose and treat endometriosis.

There is still need for improved diagnostic methods, especially for contrast agents for optical diagnosis of endometriosis in an early stage with good reliability. We have surprisingly discovered that the use of the combination of optical imaging methods and new contrast agents fulfill these requirements.

Summary of the invention

In view of the needs of the art the present invention provides a contrast agent for optical imaging with affinity for an abnormally expressed biological target associated with endometriosis.

The invention is also described in the claims.

The following definitions will be used throughout the document:

Contrast agent: Molecular moiety used for enhancement of image contrast *in vivo* comprising at least one element that interacts with light in the ultraviolet, visible or near infrared part of the electromagnetic spectrum.

Optical imaging: Any method that forms an image for diagnosis of disease, follow up of disease development or for follow up of disease treatment based on interaction with light in the electromagnetic spectrum from ultraviolet to near infrared radiation. Optical imaging includes all methods from direct visualization without use of any device and use of devices such as various scopes, catheters and optical imaging equipment, for example computer based hardware for tomographic presentations.

Diagnosis: In the context of this invention, diagnosis includes screening of selected populations, early detection, biopsy guidance, characterisation, staging, grading, therapy efficacy monitoring, long-term follow-up of relapse and surgical guidance.

Overexpressed target: A receptor, an enzyme or another molecule or chemical entity that is present in a higher amount in diseased tissue than in normal tissue.

Downregulated target: A receptor, an enzyme or another molecule or chemical entity that is present in a lower amount in CRC tissue than in normal tissue.

Detailed description of the invention

A first aspect of the present invention is contrast agents for optical imaging of endometriosis.

The contrast agent has affinity for an abnormally expressed target associated with endometriosis. By abnormally expressed, is meant that the target is either overexpressed or downregulated.

Endometriosis tissue containing a downregulated target may be identified by a low amount of bound imaging agent compared to normal tissue. In this situation, the amount of imaging agent should be less than 50 % of that in normal tissue, preferably less than 10 %.

Preferably, the contrast agent, according to the present invention, has affinity for an overexpressed target associated with endometriosis. Preferred targets are those targets that are more than 50 % abundant in endometriotic tissue than in surrounding tissue. More preferred targets are those targets that are more than two times abundant in endometriotic tissue than in surrounding tissue. The most preferred targets are those targets that are more than 5 times abundant in endometriotic tissue than in surrounding tissue.

Preferred targets are receptors, enzymes, nucleic acids, proteins, lipids, other macromolecules such as for example lipoproteins and glycoproteins. The targets may be located in the vascular system, in the extracellular space, associated with cell membranes or located intracellularly.

The following biological targets are among the preferred targets for contrast agents for optical imaging of endometriosis:

Angiogenesis targets:

Vascular endothelial growth factor, integrins (β_1-integrins, integrin alpha$_v$beta$_3$) and matrix metalloproteases.

Mainly upregulated during the secretory phase: matrix metalloproteinase 7

Receptors:
Estrogen receptors, Progesterone receptors, interleukin-1 receptor.

Mainly upregulated during the secretory phase: galectin 6-binding protein.

Adhesion molecules:
ICAM-1, CD44; cadherins such as E-cadherin, N-cadherin, P-cadherin and cadherin 11

Extracellular matrix proteins:
Tenascin, Osteopontin, Fibulin 1, Proteoglycan 4.

Enzymes:
Endothelial nitric oxide synthase, Cathepsin H, cathepsin S, Superoxide dismutase, Aromatase, Protein kinases, especially extracellular signal regulated kinase (ERK), HMG CoA reductase, Tyrosine kinases, Reductase, Protein farnesyltransferase, 17ß-hydroxysteroid dehydrogenase, cyclooxygenase-2, Xanthine oxidase, membrane-associated neutral endopeptidase (CD10), catalase and MMPs.

Mainly upregulated during the proliferative phase:
ribonuclease, phytanoyl-CoA hydroxylase, pyrroline 5-carboxylate reductase.

Mainly upregulated during the secretory phase:
palmitoyl-protein thioesterase 1, ubiquitin-conjugating enzyme E2N, malate dehydrogenase 1, aldehyde dehydrogenase 1, sterol-C5-desaturase, IGF-binding serine protease, α-L-fucosidase, glycogenin.

Oncogenes and neoplasia-related proteins:
c-myc, c-erb-B2, nm23, Hepatocellular carcinoma-associated antigen 112, acute lymphoblastic leukemia antigen (CD10) and p53.

Mainly upregulated during the proliferative phase: Src-like adaptor protein, Ras suppressor protein 1.

Mainly upregulated during the secretory phase:
neuroblastoma suppressor 1.

Cytokines and similar signal proteins:
Interleukin-6, monocyte chemotactic protein-1, Transforming growth factor, IgE-dependent histamine-releasing factor

Mainly upregulated during the proliferative phase:
Small inducible cytokine A4.

Proteins of the immune system:
C3 complement, complement component 1S subcomponent, Major histocompatibility antigens, class II, particularly DP α1, DQ α1, DR α, DQ β1, DR β and major histocompatibility complex class 1C, Ig-lambda light chain, Ig H chain G-E-A region gamma-2 constant region.

Mainly upregulated during the secretory phase:
complement component 3, properdin, complement component 1s, complement component 1r, complement component 2, Major histocompatibility antigens (class I F).

Cytoskeletal proteins:
β-actin, α_2 actin, vimentin

Mainly upregulated during the proliferative phase:
Actin-related protein 2/3 complex (subunit 1A), myosin regulatory light chain 2 (smooth muscle isoform), tropomyosin 1, ß-actin.

Transport proteins:
Folate binding proteins, Haptoglobin.

Mainly upregulated during the proliferative phase:
lactotransferrin, cellular retinol-binding protein, lysosomal H^+-transporting ATP-ase, potassium voltage-gated channel (shaker-related subfamily, member 5).

Mainly upregulated during the secretory phase: heme-binding protein.

Ribosomal proteins:
40S ribosomal protein S23.
Mainly upregulated during the proliferative phase:
ribosomal protein L11, ribosomal protein S11, ubiquitin A-52 residue ribosomal protein fusion product 1,
mainly upregulated during the secretory phase:
ribosomal protein S23.

Others:
CD14 (lipopolysaccharide binding protein), CD43, CD45, endoglin, Endometrial bleeding-associated factor (LEFTY-A), Arachidonate 5-lipooxygenase-activating protein,

Mainly upregulated during the proliferative phase:
CDC10, S100 calcium-binding protein A13, Tax interaction protein 1, GDP dissociation inhibitor 2, chimaerin 2.

Mainly upregulated during the secretory phase:
CCAAT/enhancer binding protein, epididymal secretory protein, low-density lipoprotein-related protein-associated protein 1, cyclin D2, caveolin 1, cellular repressor of E1A-stimulated genes, G-rich RNA sequence binding factor 1

Downregulated proteins:
Angiogenesis targets:
Mainly downregulated during the proliferative phase:
cysteine-rich angiogenic inducer.

Receptors:
Arginine-vasopressin receptor 1A
Mainly downregulated during the proliferative phase:
G protein-coupled receptor RDC1.

Mainly downregulated during the secretory phase:
formyl peptide receptor-like 1

Adhesion molecules:
Mainly downregulated during the secretory phase:
R-cadherin (cadherin 4), vascular endothelial junction-associated molecule,

Extracellular matrix proteins:
Mainly downregulated during the proliferative phase:
Laminin ß3.

Mainly downregulated during the secretory phase:
crystallin αB

Enzymes:
Arginase, mitogen-activated protein kinase kinase 3, cytochrome P450 2C18, glycerol kinase, serum/gucocorticoid regulated kinase, long-chain fatty acid coenzyme A ligase 5, RNA helicase.

Mainly downregulated during the proliferative phase:
carbonic anhydrase XII, pantetheinase (vanin 1)

Mainly downregulated during the secretory phase:
glutathione peroxidase, monoamine oxidase, histone deacetylase, phosphatidylserine decarboxylase, serine/threonine kinase 19, myo-inositol-monophosphatase, glycogen phosphorylase, alkylglycerone phosphate synthase, creatine kinase, phenylalanyl-tRNA synthetase ß-subunit, cullin 3.

Oncogenes and neoplasia-related proteins:
Retinoblastoma-binding protein 6, BCL2-associated athanogene 3, RAS-dexamethason-indcuced 1,

Mainly downregulated during the proliferative phase:
N-myc (downstream regulated)
Mainly downregulated during the secretory phase:
growth arrest and DNA-damage-inducible protein-α, retinoblastoma-like 1

Cytokines and similar signal proteins:
lymphotoxin-α, neurotensin

Mainly downregulated during the proliferative phase:
cytokine subfamily A member 20.

Mainly downregulated during the secretory phase:
cytokine subfamily B member 14, colony-stimulating factor 3, interferon-related developmental regulator 1,

Proteins of the immune system:
Mainly downregulated during the secretory phase:
C4-binding protein-α,

Cytoskeletal proteins:
Mainly downregulated during the secretory phase:
Actin γ2, actinin α4, plectin 1,

Transport proteins:
Mainly downregulated during the secretory phase:
solute carrier family 3 member 1

Ribosomal proteins:
Mainly downregulated during the secretory phase:
mitochondrial ribosomal protein S2.

Others:
Kruppel-like factor 5, GATA-binding protein 2, heat shock protein 105 kDa, non-histone chromosomal proteins I and Y, oviducal glycoprotein 1 (oviductin), DNAJ-like HSP 2, DNAJ (Hsp40) homolog subfamily B member 1, stromal antigen 2, Heat shock protein 70, Heat shock protein 27.

Mainly downregulated during the proliferative phase:
Zinc finger protein 216, adipose differentiation-related protein, heterogeneous nuclear ribonucleoprotein A1, stanniocalcin 1, Golgi membrane protein GP73

Mainly downregulated during the secretory phase:
anaphase-promoting complex subunit 4, H1 histone family member 0, t-complex-associated-testis-expressed 1-like, regulator of G-protein signalling 9, Islet cell autoantigen 1, metal-regulatory transcription factor 1, heat shock protein 70 kDa protein 1A, thyrotropin-releasing hormone, metallothionein 1E.

Among the most preferred targets for contrast agents for optical imaging of endometriosis are angiogenesis targets, adhesion molecules, estrogen receptors, progesterone receptors, Cathepsin H and Cathepsin S, aromatase, reductase, CD10, endoglin, haptoglobin and cyclin D2.

Generally, any targets that have been identified as possible targets for agents for treatment of endometriosis might be potential targets also in optical imaging.

The contrast agents, according to the present invention, comprise a targeting moiety that binds to an abnormally expressed target in endometriotic tissue, and an optical reporter.

The preferred contrast agents are molecules with relatively low molecular weights. The molecular weight of preferred contrast agents is below 10000 Daltons, more preferably below 7000 Daltons.

Thus viewed from one aspect the present invention provides a contrast agent of formula I:

V-L-R (I)

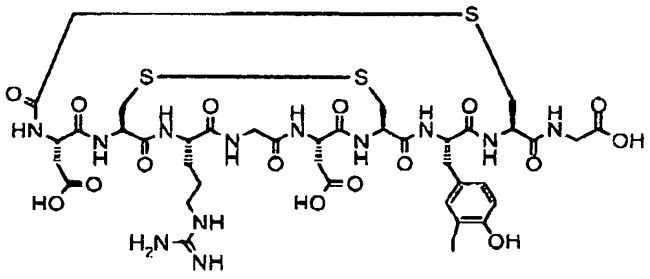
wherein V is one or more vector moieties having affinity for one or more abnormally expressed target in endometriosis, L is a linker moiety or a bond and R is one or more reporter moieties detectable in optical imaging.

Hence, the contrast agents of the present invention comprise at least one optical reporter molecule linked to a molecule sub-unit (vector) that binds to an abnormally expressed target in endometriotic tissue.

The agents of formula I have three characteristic components: a vector (V); a linker (L); and a reporter (R). The vector must have the ability to target the contrast agent to a region of endometriosis, the reporter must be detectable in an optical imaging procedure and the linker must couple vector to reporter, at least until the reporter has been delivered to the region of endometriosis and preferably until the imaging procedure has been completed.

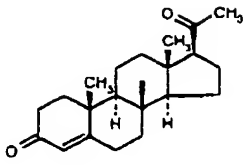
The vector can for example be selected from the following group of compounds: peptides, peptidomimetics, oligonucleotides, oligosaccharides, fat-related compounds and traditional organic molecules. The targeting part of the contrast agent should preferably have a molecular weight of less than 4500 Daltons and more preferably less than 2500 Daltons. Below are some examples of vectors having affinity for endometriosis related abnormally expressed targets:

Vectors for angiogenesis targets:
Vector I: An RGD-peptide such as e.g.



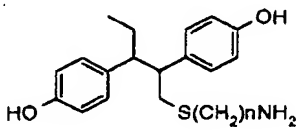
Vector for progesterone receptors:

Vector II: Progesterone or a derivative thereof



Vectors for estrogen receptors:

Vector III: Estrogen, estrogen mimetics or a derivative thereof, e.g.

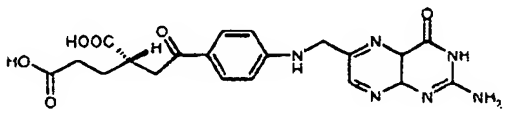


n = 2-7

Coupling takes place via the amine group

Vector for folate binding proteins:

Vector IV: Folate or a derivative thereof



A wide variety of linkers can be used. The linker component of the contrast agent is at its simplest a bond between the vector and the reporter moieties. In this aspect the reporter part of the molecule is directly bound to the molecule sub-unit that binds to the abnormally expressed target. More generally however the linker will provide a mono- or multi-molecular skeleton covalently or non-covalently linking one or more vectors to one or more reporters, e.g. a linear, cyclic, branched or reticulate molecular skeleton, or a molecular aggregate, with in-built or pendant groups which bind covalently or non-covalently, e.g. coordinatively, with the vector and reporter moieties. The linker group can be relatively large in order to build into the contrast agent optimal size or optimal shape or simply to improve the binding characteristics for the contrast agent to the abnormally expressed target in endometriotic tissue.

Thus linking of a reporter unit to a desired vector may be achieved by covalent or non-covalent means, usually involving interaction with one or more functional groups located on the reporter and/or vector. Examples of chemically reactive functional groups which may be

employed for this purpose include amino, hydroxyl, sulfhydroxyl, carboxyl and carbonyl groups, as well as carbohydrate groups, vicinal diols, thioethers, 2-aminoalcohols, 2-aminothiols, guanidinyl, imidazolyl and phenolic groups.

Covalent coupling of reporter and vector may therefore be effected using linking agents containing reactive moities capable of reaction with such functional groups. Examples of reactive moieties capable of reaction with sulfhydryl groups include a-haloacetyl compounds of the type X-CH_2CO- (where X=Br, Cl or I) which show particular reactivity for sulfhydryl groups but which can also be used to modify imidazolyl, thioether, phenol and amino groups. N- Maleimide derivatives are also considered selective towards sulfhydryl groups, but may additionally be useful in coupling to amino groups under certain conditions. Reagents such as 2-iminothiolane which introduce a thiol group through conversion of an amino group, may be considered as sulfhydryl reagents if linking occurs through the formation of disulphide bridges. Thus reagents which introduce reactive disulphide bonds into either the reporter or the vector may be useful, since linking may be brought about by disulphide exchange between the vector and reporter; examples of such reagents include Ellman's reagent (DTNB), 4,41-dithiodipyridine, methyl- 3-nitro-2-pyridyl disulphide and methyl-2-pyridyl disulphide.

Examples of reactive moieties capable of reaction with amino groups include alkylating and acylating agents. Representative alkylating agents include:
i)α-haloacetyl compounds, which show specificity towards amino groups in the absence of reactive thiol groups and are of the type X-CH_2CO- (where X=Cl, Br or I);
ii) N-maleimide derivatives, which may react with amino groups either through a Michael type reaction or through acylation by addition to the ring carbonyl group;
iii) aryl halides such as reactive nitrohaloaromatic compounds;
iv) alkyl halides;
v) aldehydes and ketones capable of Schiff's base formation with amino groups, the adducts formed usually being stabilised through reduction to give a stable amine;
vi) epoxide derivatives such as epichlorohydrin and bisoxiranes,which may react with amino, sulfhydryl or phenolic hydroxyl groups;
vii) chlorine-containing derivatives of s-triazines, which are very reactive towards nucleophiles such as amino, sufhydryl and hydroxy groups;
viii) aziridines based on s-triazine compounds detailed above, which react with nucleophiles such as amino groups by ring opening;
ix) squaric acid diethyl esters
X) α-haloalkyl ethers, which are more reactive alkylating agents than normal alkyl halides because of the activation caused by the ether oxygen atom.

Representative amino-reactive acylating agents include:
i) isocyanates and isothiocyanates, particularly aromatic derivatives, which form stable urea and thiourea derivatives respectively and have been used for protein crosslinking;
ii) sulfonyl chlorides, which may be useful for the introduction of a fluorescent reporter group into the linker; iii) Acid halides;
iv) Active esters such as nitrophenylesters or N- hydroxysuccinimidyl esters;
v) acid anhydrides such as mixed, symmetrical or N- carboxyanhydrides;
vi) other useful reagents for amide bond formation;
vii) acylazides, e.g. wherein the azide group is generated from a preformed hydrazide derivative using sodium nitrite; viii) azlactones attached to polymers such as bis- acrylamide;
ix) Imidoesters , which form stable amidines on reaction with amino groups.

Carbonyl groups such as aldehyde functions may be reacted with weak protein bases at a pH such that nucleophilic protein side-chain functions are protonated. Weak bases include 1,2-aminothiols such as those found in N-terminal cysteine residues, which selectively form stable 5-membered thiazolidine rings with aldehyde groups. Other weak bases such as phenyl hydrazones may be used. Aldehydes and ketones may also be reacted with amines to form Schiff's bases, which may advantageously be stabilised through reductive amination. Alkoxylamino moieties readily react with ketones and aldehydes to produce stable alkoxamines.

Examples of reactive moieties capable of reaction with carboxyl groups include diazo compounds such as diazoacetate esters and diazoacetamides, which react with high specificity to generate ester groups. Carboxylic acid modifying reagents such as carbodiimides, which react through O-acylurea formation followed by amide bond formation, may also usefully be employed; linking may be facilitated through addition of an amine or may result in direct vector-receptor coupling. Useful water soluble carbodiimides include 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimide (CMQ and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Other useful carboxylic acid modifying reagents include isoxazolium derivatives such as Woodwards reagent K; chloroformates such as p-nitrophenylchloroformate; carbonyldiimidazoles such as 1,1-carbonyldiimidazole; and N-carbalkoxydihydroquinolines such as N-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline. Other potentially useful reactive moieties include vicinal diones such as p-phenylenediglyoxal, which may be used to react with guanidinyl groups; and diazonium salts, which may undergo electrophilic substitution reactions. Compounds are readily prepared by treatment of aryl diamines with sodium nitrite in acidic solutions.

It will be appreciated that functional groups in the reporter and/or vector may if desired be converted to other functional groups prior to reaction, e.g. to confer additional reactivity or selectivity. Examples of methods useful for this purpose include conversion of amines to

carboxylic acids using reagents such as dicarboxylic anhydrides; conversion of amines to thiols using reagents such as N-acetylhomocysteine thiolactone, S- acetylmercaptosuccinic anhydride, 2-iminothiolane or thiol-containing succinimidyl derivatives; conversion of thiols to carboxylic acids using reagents such as a-haloacetates; conversion of thiols to amines using reagents such as ethylenimine or 2-bromoethylamine; conversion of carboxylic acids to amines using reagents such as carbodiimides followed by diamines; and conversion of alcohols to thiols using reagents such as tosyl chloride followed by transesterification with thioacetate and hydrolysis to the thiol with sodium acetate.

The reporter moieties in the contrast agents of the invention may be any moiety capable of detection either directly or indirectly in an optical imaging procedure. The reporter might be a light scatterer (e.g. a coloured or uncoloured particle), a light absorber or a light emitter. More preferably the reporter is a dye such as a chromophore or a fluorescent compound. The dye part of the contrast agent can be any dye that interacts with light in the electromagnetic spectrum with wavelenghts from the ultraviolet light to the near infrared. Preferably the contrast agent of the invention has fluorescent properties.

Preferred organic chromophoric and fluorophoric reporters include groups having an extensive delocalized electron system, eg. cyanines, merocyanines, indocyanines, phthalocyanines, naphthalocyanines, triphenylmethines, porphyrins, pyrilium dyes, thiapyriliup dyes, squarylium dyes, croconium dyes, azulenium dyes, indoanilines, benzophenoxazinium dyes, benzothiaphenothiazinium dyes, anthraquinones, napthoquinones, indathrenes, phthaloylacridones, trisphenoquinones, azo dyes, intramolecular and intermolecular charge-transfer dyes and dye complexes, tropones, tetrazines, bis(dithiolene) complexes, bis(benzene-dithiolate) complexes, iodoaniline dyes, bis(S,O-dithiolene) complexes. Fluorescent proteins, such as green fluorescent protein (GFP) and modifications of GFP that have different absorption/emission properties are also useful. Complexes of certain rare earth metals (e.g., europium, samarium, terbium or dysprosium) are used in certain contexts, as are fluorescent nanocrystals (quantum dots).

Particular examples of chromophores which may be used include fluorescein, sulforhodamine 101 (Texas Red), rhodamine B, rhodamine 6G, rhodamine 19, indocyanine green, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, Marina Blue, Pacific Blue, Oregon Green 488, Oregon Green 514, tetramethylrhodamine, and Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and Alexa Fluor 750.

Particularly preferred are dyes which have absorption maxima in the visible or near infrared region, between 400 nm and 3 μm, particularly between 600 and 1300 nm.

Several relevant targets for endometriosis are enzymes. A contrast agent for optical imaging of endometriosis for targeting an enzyme can be an enzyme contrast agent substrate that can be transformed to a contrast agent product possessing different pharmacokinetic and/or pharmacodynamic properties from the contrast agent substrate. In this embodiment the invention provides contrast agent substrates having affinity for an abnormally expressed enzyme, wherein the contrast agent substrate changes pharmacodynamic and/or pharmacokinetic properties upon a chemical modification into a contrast agent product in a specific enzymatic transformation, and thereby enabling detection of areas of disease upon a deviation in the enzyme activity from the normal. Typical differences in pharmacodynamic and/or pharmacokinetic properties can be binding properties to specific tissue, membrane penetration properties, protein binding and solubility issues.

Alternatively, if the abnormally expressed target for diagnosis of endometriosis is an enzyme, the contrast agent for optical imaging can be a dye molecule that directly binds to the enzyme. The contrast agent will have affinity for the abnormally expressed enzyme, and this may be used to identify tissue or cells with increased enzymatic activity.

In a further aspect of the invention the contrast agent changes dye characteristics as a result of an enzymatic transformation. For example, a fluorescent dye reporter of the contrast agent is quenched (no fluorescence) by associated quencher groups, until an enzymatic cleavage takes place, separating the dye from the quencher groups and resulting in fluorescence at the site of the abnormally expressed enzyme.

Another aspect of this part of the invention is that the dye may change colour, as e.g. a change in absorption and/or emission spectrum, as a result of an enzymatic transformation.

If the abnormally expressed target for diagnosis of endometriosis is a receptor or another non-catalytical target, the contrast agent for optical imaging can bind directly to the target and normally not change the dye characteristics.

Another aspect of the invention is contrast agents for optical imaging of endometriosis characterized by having affinity for more than one abnormally expressed target related to the disease. Such contrast agents can have two or more different vectors or molecular subunits that target two or more different abnormally expressed targets.

Another possibility according to the present invention is that the contrast agent has one vector that is able to bind to more than one abnormally expressed target in endometriosis.

A contrast agent according to the present invention might also have more than one vector of same chemical composition that binds to the abnormally expressed biological target.

Another aspect of the present invention is contrast agents for optical imaging of endometriosis characterized in that the contrast agent comprises more than one dye molecular sub-unit. These dye sub-units might be similar or different from a chemical point of view. Preferred contrast agents have less than 6 dye molecular sub-units.

The preferred contrast agents of the present invention are soluble in water. This means that the preferred contrast agents have a solubility in water at pH 7.4 of at least 1 mg/ml.

The contrast agents of the present invention can be identified by random screening, for example by testing of affinity for abnormally expressed targets of a library of dye labeled compounds either prepared and tested as single compounds or by preparation and testing of mixture of compounds (a combinatorial approach).

The contrast agents of the present invention can also be identified by use of technology within the field of intelligent drug design. One way to perform this is to use computer-based techniques (molecular modelling or other forms of computer-aided drug design) or use of knowledge about natural and exogenous ligands (vectors)for the abnormally expressed targets. The sources for exogenous ligands can for example be the chemical structures of therapeutic molecules for targeting the same target. One typical approach here will be to bind the dye chemical sub-unit to the targeting vector so that the binding properties of the vector are not reduced. This can be performed by linking the dye at the far end, away from the pharmacophore centre (the active targeting part of the molecule).

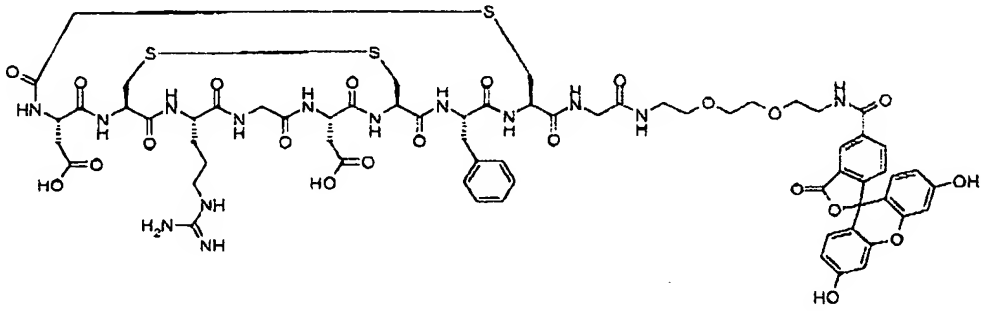
The contrast agents of the invention are preferably not endogenous substances alone. Some endogenous substances, for instance estrogen, have certain fluorescent properties in themselves, but they are not likely to be sufficient for use in optical imaging. Endogenous substances combined with an optical reporter however, falls within the contrast agents of the invention.

The contrast agents will be useful with optical imaging modalities and measurement techniques including, but not limited to: luminescence imaging; endoscopy; fluorescence endoscopy; optical coherence tomography; transmittance imaging; time resolved transmittance imaging; confocal imaging; nonlinear microscopy; photoacoustic imaging; acousto-optical imaging; spectroscopy; reflectance spectroscopy; interferometry; coherence interferometry; diffuse optical tomography and fluorescence mediated diffuse optical tomography (continuous wave, time domain and frequency domain systems), and measurement of light scattering, absorption, polarisation, luminescence, fluorescence lifetime, quantum yield, and quenching.

Some examples on contrast agent molecules for optical imaging of endometriosis according to the invention are shown below with some accompanied suggested synthetic routes:

Contrast agents with affinity for angiogenesis:

Compound 1:

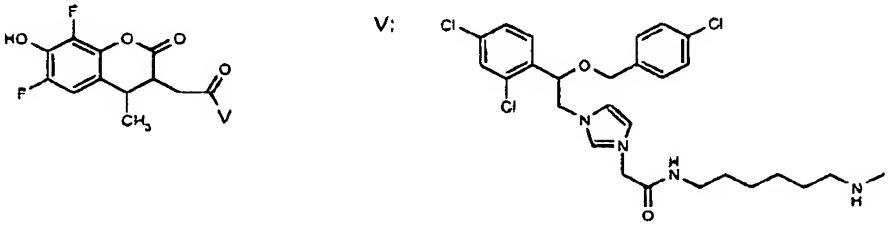


Reporter: RGD-peptide

Linker: PEG-moiety

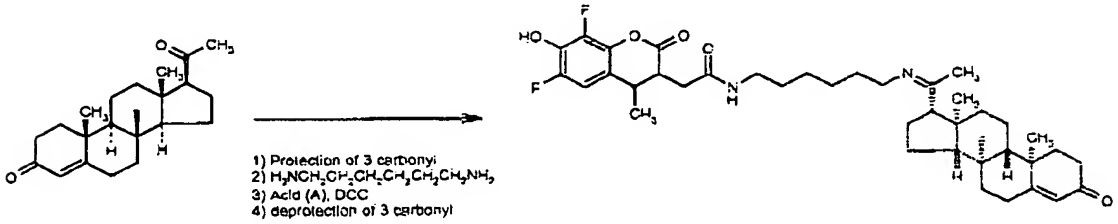
Reporter: Fluorescein

Compound II:

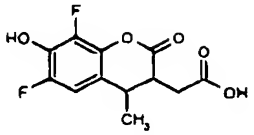


Contrast agent with affinity for progesteron receptors:

Compound III:

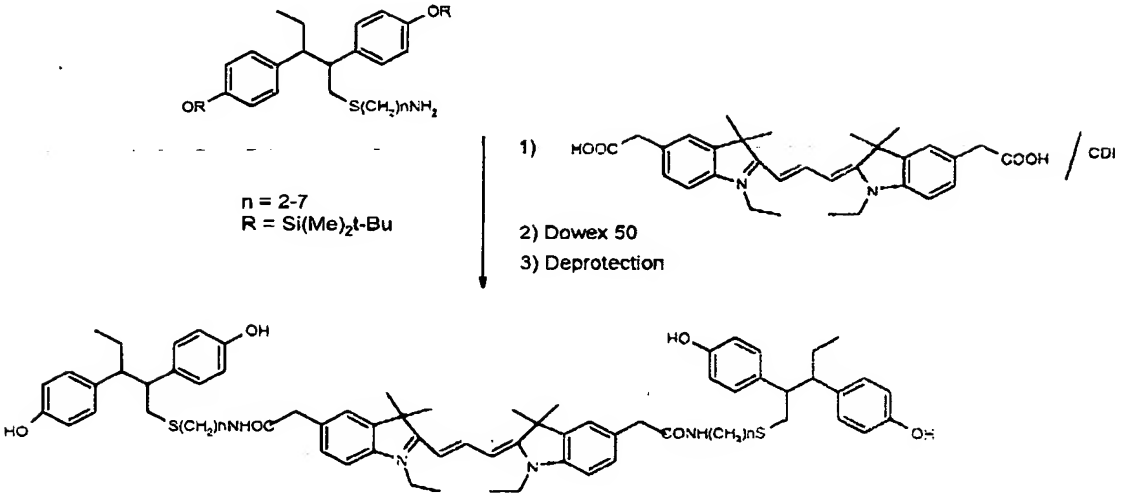


Acid (A):

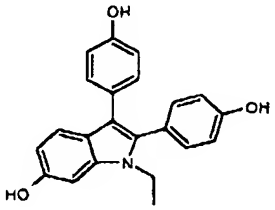


Contrast agent with affinity for estrogen receptors:

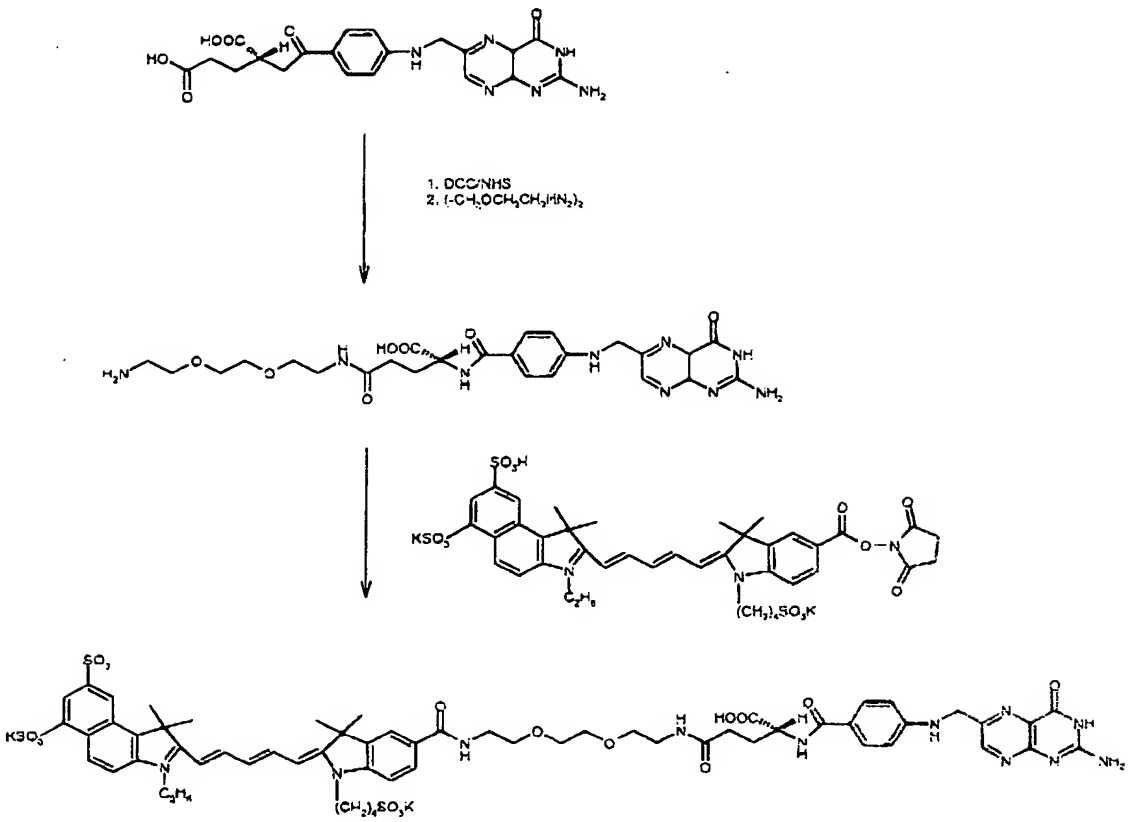
Compound IV:



Compound V:



Contrast agent with affinity for folate binding proteins:
Compound VI:



This complex comprising a NIR dye, a PEG moiety and a folate vector has been described in Bioconjugate Chem., Vol. 14, Ni.3, 2003, but not in the context of optical imaging of endometriosis.

A further embodiment is use of contrast agents of the invention for optical imaging of endometriosis, that is, for diagnosis, for follow up of the progress in endometriosis development or for follow up the treatment of endometriosis.

Still another embodiment of the invention is a method of optical imaging for diagnosis of endometriosis using the contrast agents as described.

Still another embodiment of the invention is a method of optical imaging to follow up the progress of endometriosis development and to follow up the treatment of endometriosis.

One aspect of these methods is to administer the present contrast agents and follow the accumulation and elimination directly visually during surgery. Another aspect of these

methods is to administer the present contrast agents and perform visual diagnosis through a laparoscope.

Still another aspect of the present invention is to administer the present contrast agents and perform the image diagnosis using computerized equipment as for example a tomograph.

Still another embodiment of the invention is use of a contrast agent as described for the manufacture of a diagnostic agent for use in a method of optical imaging of endometriosis involving administration of said diagnostic agent to an animate subject and generation of an image of at least part of said body.

Still another embodiment of the invention is pharmaceutical compositions comprising one or more contrast agents as described or pharmaceutically acceptable salts thereof for optical imaging for diagnosis of endometriosis, for follow up progress of endometriosis development or for follow up the treatment of endometriosis. The diagnostic agents of the present invention may be formulated in conventional pharmaceutical or veterinary parenteral administration forms, e.g. suspensions, dispersions, etc., for example in an aqueous vehicle such as water for injections. Such compositions may further contain pharmaceutically acceptable diluents and excipients and formulation aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc. The most preferred formulation is a sterile solution for intravascular administration or for direct injection into area of interest. Where the agent is formulated in a ready-to-use form for parenteral administration, the carrier medium is preferably isotonic or somewhat hypertonic.

The dosage of the optical diagnostic agents of the invention will depend upon the clinical indication, choice of contrast agent and method of administration. In general, however dosages will be between 10μg and 5 grams for an adult human.

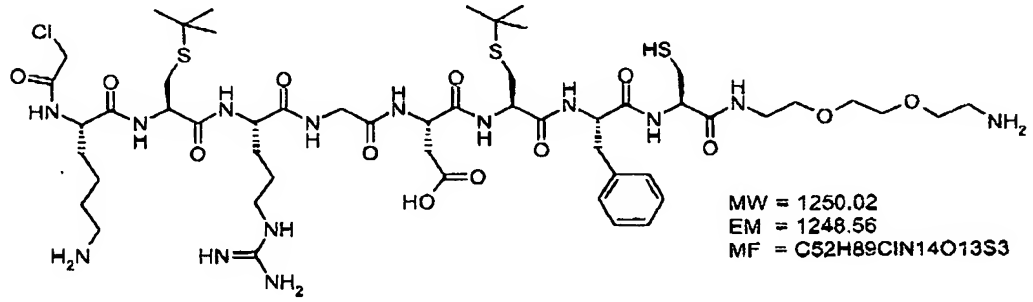
While the present invention is particularly suitable for methods involving parenteral administration of the contrast agent, e.g. into the vasculature or directly into an organ of muscle tissue, intravenous administration being especially preferred, it is also applicable where administration is not via a parenteral route, e.g. where administration is transdermal, nasal, sub-lingual or is into an externally voiding body cavity, e.g. the gi tract, the bladder, the uterus or the vagina. The present invention is deemed to extend to cover such administration.

The following examples are illustrative only and not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

Examples

Example 1. Contrast agent with affinity for angiogenesis:
RGD-vector with two Cy5.5 units

Step 1) Synthesis of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-PEG-NH_2 (I)

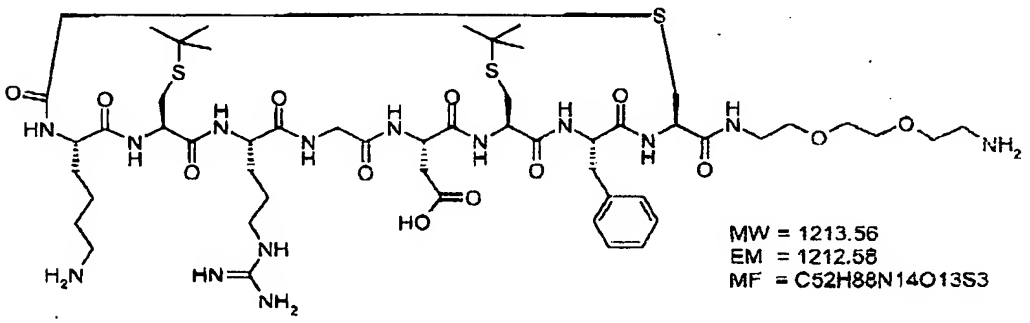


The peptide was synthesised on a ABI 433A automatic peptide synthesiser starting with O-Bis-(aminoethyl)ethylene glycol trityl resin on a 0.25 mmol scale using 1 mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling. The N-terminus was chloroacetylated using a solution of chloroacetic anhydride in DMF for 30 min.

The simultaneous removal of peptide and side-chain protecting groups (except tBu) from the resin was carried out in TFA containing TIS (2.5 %) and H_20 (2.5 %) for two hours.

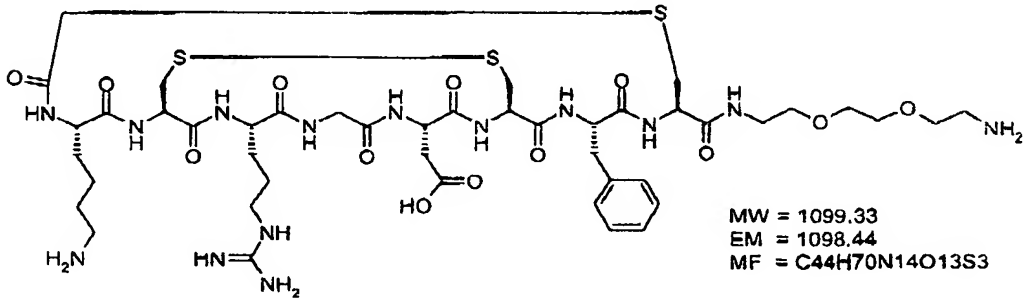
After work-up 330 mg of crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3µ C18 (2) 50 x 4.6 mm; flow, 1 mL/min; detection, UV 214 nm; product retention time, 6.78 min). Further product characterisation was carried out using mass spectrometry: Expected, MH^+ at 1249.6, found, at 1249.4).

Step 2) Synthesis of cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-PEG-NH_2 (II)



330 mg of compound I was dissolved in water/acetonitrile (1:1, 500 mL). The mixture was adjusted to pH 8 with ammonia solution and stirred for 4 hours. Acetonitrile was removed by evaporation *in vacuo* and the residue lyophilised affording crude monocyclic intermediate as a white powder.

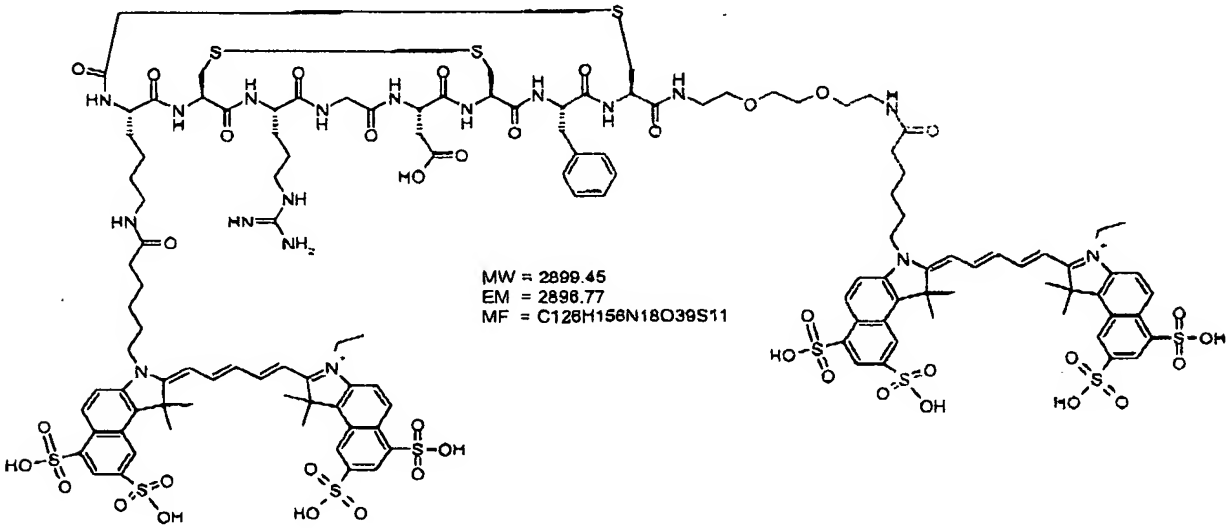
Step 3) Synthesis of [Cys^{2-6}] cyclo[CH_2CO-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-PEG-NH_2 (III)



217 mg of compound II was treated with a solution of DMSO (15 mL) and TFA (285 mL) for 30 min following which the TFA was removed *in vacuo* and the peptide precipitated by the addition of diethyl ether.

Purification by preparative HPLC (Phenomenex Luna 10µ C18 (2) 250 x 21.2 mm column) of the crude material (181 mg) was carried out using 5-50 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation pure bicyclic intermediate was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3µ C18 (2) 50 x 4.6 mm; flow, 1 mL/min; detection, UV 214 nm; product retention time, 8.11 min). Further product characterisation was carried out using mass spectrometry: Expected, MH^+ at 1099.4, found, at 1099.4).

Step 4) Synthesis of [Cys^{2-6}] cyclo[CH_2CO-Lys(Cy5.5)-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-PEG-Cy5.5 (IV)

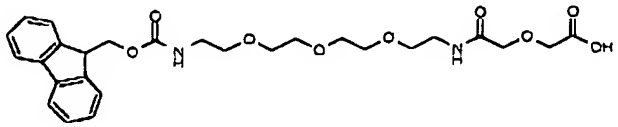


1.6 mg of compound III, 8.9 mg of Cy5.5 NHS ester and 4 µL of N-methylmorpholine was dissolved in dimethylformamide (0.5 mL). The mixture was stirred for 3 days.

Purification by preparative HPLC (Phenomenex Luna 5µ C18 (2) 250 x 10 mm column) of the reaction mixture was carried out using 5-50 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 30 min at a flow rate of 10 mL/min. After lyophilisation 1.6 mg of pure material was obtained (Analytical HPLC: Gradient, 10-60 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3µ C18 (2) 50 x 4.6 mm; flow, 1 mL/min; detection, UV 214 nm; product retention time, 6.42 min). Further product characterisation was carried out using mass spectrometry: Expected, $(MH_2)^{2+}$ at 1448.4, found, at 1448.3).

Example 2: Contrast agent with affinity for angiogenesis: RGD-vector linked to Cy 5.5. Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(Cy5.5)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-PEG-NH_2

Step a) Synthesis of 17-(Fmoc-amino)-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid



This building block is coupled to the solid-phase using Fmoc chemistry. The coupled form of this building block will be referred to in short as PEG.

1,11-Diazido-3,6,9-trioxaundecane

A solution of dry tetraethylene glycol (19.4 g, 0.100 mol) and methanesulphonyl chloride (25.2 g, 0.220 mol) in dry THF (100 ml) was kept under argon and cooled to 0 °C in an ice/water bath. To the flask was added a solution of triethylamine (22.6 g, 0.220 mol) in dry THF (25 ml) dropwise over 45 min. After 1 hr the cooling bath was removed and stirring was continued for 4 hrs. Water (60 ml) was added. To the mixture was added sodium hydrogencarbonate (6 g, to pH 8) and sodium azide (14.3 g, 0.220 mmol), in that order. THF was removed by distillation and the aqueous solution was refluxed for 24 h (two layers formed). The mixture was cooled and ether (100 ml) was added. The aqueous phase was saturated with sodium chloride. The phases were separated and the aqueous phase was extracted with ether (4 x 50 ml). Combined organic phases were washed with brine (2 x 50 ml) and dried ($MgSO_4$). Filtration and concentration gave 22.1 g (91%) of yellow oil. The product was used in the next step without further purification.

11-Azido-3,6,9-trioxaundecanamine

To a mechanically, vigorously stirred suspension of 1,11-diazido-3,6,9-trioxaundecane (20.8 g, 0.085 mol) in 5% hydrochloric acid (200 ml) was added a solution of triphenylphosphine (19.9 g, 0.073 mol) in ether (150 ml) over 3 hrs at room temperature. The reaction mixture was stirred for additional 24 hrs. The phases were separated and the aqueous phase was extracted with dichloromethane (3 x 40 ml). The aqueous phase was cooled in an ice/water bath and pH was adjusted to ca 12 by addition of KOH. The product was extracted into dichloromethane (5 x 50 ml). Combined organic phases were dried ($MgSO_4$). Filtration and evaporation gave 14.0 g (88%) of yellow oil. Analysis by MALDI-TOF mass spectroscopy (matrix: α-cyano-4-hydroxycinnamic acid) gave a M+H peak at 219 as expected. Further characterisation using ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopy verified the structure.

17-Azido-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid

To a solution of 11-azido-3,6,9-trioxaundecanamine (10.9 g, 50.0 mmol) in dichloromethane (100 ml) was added diglycolic anhydride (6.38 g, 55.0 mmol). The reaction mixture was stirred overnight. HPLC analysis (column Vydac 218TP54; solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 4-16% B over 20 min; flow 1.0 ml/min; UV detection at 214 and 284 nm), showed complete conversion of starting material to a product with retention time 18.3 min. The solution was concentrated to give quantitative yield of a yellow syrup. The product was analysed by LC-MS (ES ionisation) giving $[MH]^+$ at 335 as expected. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopy was in agreement with structure
The product was used in the next step without further purification.

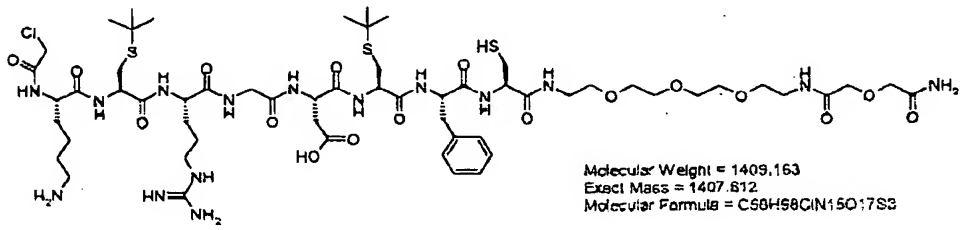
17-Amino-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid

A solution of 17-azido-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid (8.36 g, 25.0 mmol) in water (100 ml) was reduced using H_2(g)-Pd/C (10%). The reaction was run until LC-MS analysis showed complete conversion of starting material (column Vydac 218TP54; solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 4-16% B over 20 min; flow 1.0 ml/min; UV detection at 214 and 284 nm, ES ionisation giving M+H at 335 for starting material and 309 for the product). The solution was filtered and used directly in the next step.

17-(Fmoc-amino)-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid

To the aqueous solution of 17-amino-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid from above(corresponding to 25.0 mmol amino acid) was added sodium bicarbonate (5.04 g, 60.0 mmol) and dioxan (40 ml). A solution of Fmoc-chloride (7.11 g, 0.275 mol) in dioxan (40 ml) was added dropwise. The reaction mixture was stirred overnight. Dioxan was evaporated off (rotavapor) and the aqueous phase was extracted with ethyl acetate. The aqueous phase was acidified by addition of hydrochloric acid and precipitated material was extracted into chloroform. The organic phase was dried ($MgSO_4$), filtered and concentrated to give 11.3 g (85%) of a yellow syrup. The structure was confirmed by LC-MS analysis (column Vydac 218TP54; solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 40-60% B over 20 min; flow 1.0 ml/min; UV detection at 214 and 254 nm, ES ionisation giving M+H at 531 as expected for the product peak at 5,8 minutes). The analysis showed very low content of side products and the material was used without further purification.

Step b) Synthesis of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-PEG-NH_2

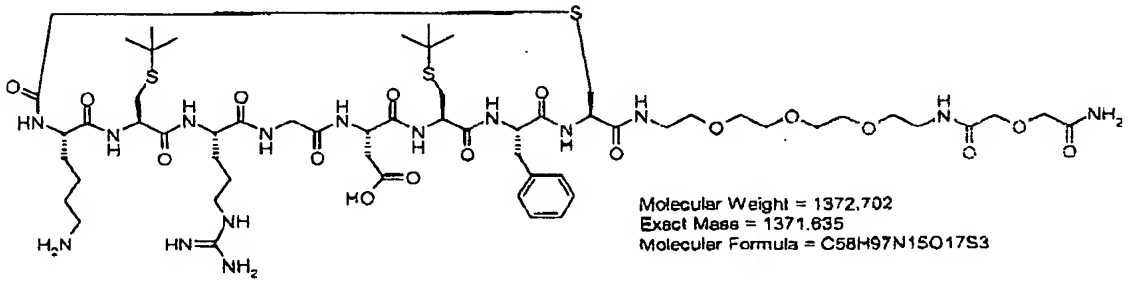


The PEG unit synthesized in step a) was coupled manually to Rink Amide AM resin, starting on a 0.25 mmol scale, mediated by HATU activation. The remaining peptide was assembled on an ABI 433A automatic peptide synthesiser using 1 mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling. N-terminal amine groups were chloroacetylated using a solution of chloroacetic anhydride in DMF for 30 min.

The simultaneous removal of peptide and side-chain protecting groups (except tBu) from the resin was carried out in TFA containing TIS (5 %), H_20 (5 %) and phenol (2.5 %) for two hours.

After work-up 322 mg of crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 μ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.37 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1409, found, at 1415).

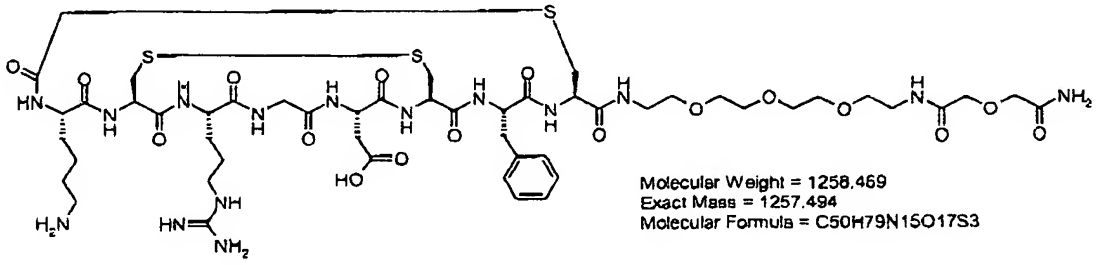
Step c) Synthesis of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-PEG-NH_2



322 mg of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-(PEG)n-NH_2 was dissolved in water/acetonitrile. The mixture was adjusted to pH 8 with ammonia solution and stirred for 16 hours. After work-up crude peptide was obtained (Analytical HPLC: Gradient, 5-

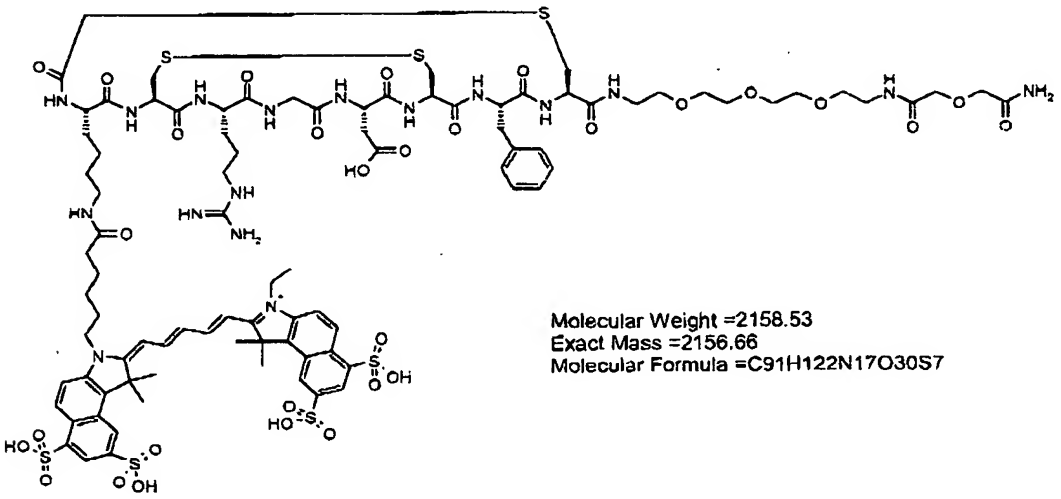
50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 μ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.22 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1373, found, at 1378).

Step d) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-PEG-NH_2



Thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-(PEG)n-NH_2 was treated with a solution of anisole (200 μL), DMSO (2 mL) and TFA (100 mL) for 60 min following which the TFA was removed in vacuo and the peptide precipitated by the addition of diethyl ether. Purification by preparative HPLC (Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm column) of 70 mg crude material was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 46 mg of pure material was obtained (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 μ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.80 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1258.5, found, at 1258.8).

Step e) Synthesis of disulfide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(Cy5.5)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-PEG-NH_2



Molecular Weight =2158.53
Exact Mass =2156.66
Molecular Formula =C91H122N17O30S7

30 mg of [Cys^{2-6}] cyclo[CH_2CO-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-PEG-NH_2, 29.4 mg of Cy5.5 NHS ester and 6 µL of N-methylmorpholine was dissolved in DMF. The mixture was protected against light and stirred over night.

Purification by preparative HPLC (Vydac 218TP1022 C18 column) of the reaction mixture was carried out using 10-50 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. Re purification by preparative HPLC (Vydac 218TP1022 C18 column using 10-50 % B, where A = 90% water, 10% ACN, 0.1% NH4OAc, B = 90% ACN, 10% water, 0.1% NH4OAc over 40 min at a flow rate of 10 mL/min was carried out. After lyophilisation 13.1 mg of pure material was obtained (Analytical HPLC: Gradient, 10-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 5 µ C18 (2) 250 x 4.6 mm; flow, 1 mL/min; detection, UV 214 nm; product retention time, 13.9 min). Further product characterisation was carried out using mass spectrometry: Expected, M+ at 2156.7, found, at 1078.8 (two charges) 2156.6.

Binding of the product of step e) to EA-Hy929 cells has been demonstrated using flow cytometry and fluorescence microscopy. The A-Hy926 cell membrane competition assay has demonstrated excellent binding properties to the vitronectin receptor ($\alpha_v\beta_3$ integrin); K_I in the nanomolar range. The compound has been shown to have no acute toxic effects in healthy mice, even at high doses.

Example 3 Contrast agent with affinity for folate binding protein. Synthesis of folic acid – fluorescin linker conjugate

Step 1
Folic acid dehydrate (1.0 g) is mixed with toluene (500 ml) and heated to 110°C. The mixture is cooled to 50°C and evaporated to dryness. Folic acid anhydrate is isolated.

Step 2
Folic acid anhydrate (441 mg, 1 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (226 mg, 1.1 mmol) are dissolved in DMF (30 ml). The mixture is cooled to 0°C and a solution of 2,2'-ethylenedioxy)bis(ethylamine) (296 mg, 2 mmol) and DMAP (30 mg) in DMF(10 ml) is added. The mixture is stirred for 1 hour at 0°C and then stirred for 72 hours at ambient temperature. The solution is evaporated and the conjugate between folic acid and the bisamide is isolated as monoamide by chromatography (silica, chloroform and methanol).

Step 3
5(6) – Carboxyfluorescein (188 mg, 0.5 mmol) and dicyclohexylcarbodiimide (113 mg, 0.55 mmol) are dissolved in DMF (20ml). The mixture is cooled to 0°C and a solution of the monoamide from step 2 above (286 mg, 0.5 mmol) and DMAP (15 mg) in DMF (5 ml) is added. The mixture is stirred for 1 hour at 0°C and then stirred for 72 hours at ambient temperature. The solution is evaporated and the final conjugate is isolated by chromatography (silica, chloroform and methanol).

Example 4 Contrast agent for mapping of matrix metalloproteinase (MMP). Synthesis of fluorescein–Cys-Gly-Pro-Leu-Gly-Lev-Leu-Ala-Arg-OH linker conjugate

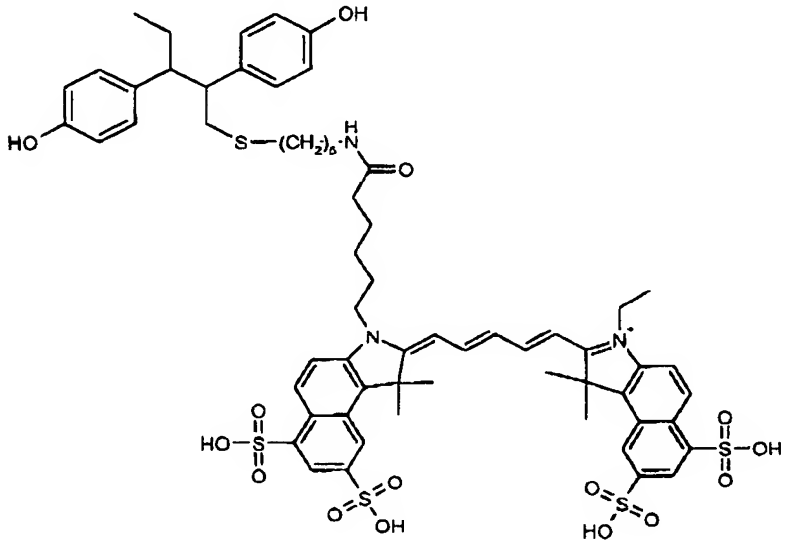
Step 1
The peptide component was synthesised on an ABI 433A automatic peptide synthesiser starting with Fmoc –Arg (Pmc) –wang resin on a 0.1mmol scale using 1mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling. An aliquot of the peptide resin was then transferred to a clean round bottom flask an N-methyl morpholine (1 mmol) in DMF (5 ml) added followed by chloroacetyl chloride (1mmol). The mixture was gently shaken until Kaiser test negative. The resin was extensively washed with DMF.

Step 2
5(6)–carboxyfluorescein (188 mg, 0.5 mmol) and dicyclohexylcarbodiimide (113 mg, 0.55 mmol) are dissolved in DMF (20 ml). The mixture is stirred for 2 hours and cooled to 0°C. A solution of hexamethylenediamide (116 mg, 1 mmol) and DMAP (30 mg) in DMF is added and the mixture is stirred at ambient temperature for 72 hours. The solution is evaporated and

the conjugate between carboxyfluorescein and hexamethylene-amine is isolated as monoamide by chromatography (silica, chloroform and methanol).

Step 3
The resin from step 1 is suspended in DMF (5 ml) and amide-amine conjugate from step 2 (0.5 mmol) pre-dissolved in DMF (5ml) containing triethylamine (0.5 mmol) is added. The mixture is heated to 50°C for 16 hours then excess reagents filtered off, following extensive washing with DMF, DCM and diethyl ether then air drying. The product is treated with TFA containing TIS (5%), H_2O (5%), and phenol (2.5%) for 2 hours.
Excess TFA is removed *in vacuo* and the peptide is precipitated by the addition of diethyl ether. The crude peptide conjugate is purified by preparative HPLC C C-18, acetonitril, TFA, water).

Example 5 Contrast agent for mapping of estrogen receptors; Cy 5.5 linked to estrogen derivative



(2R, 3S)-2,3-bis[4-[(tert-butyldimethylsilyl)oxy]-phenyl]pentyl-5-aminopenylsulfide is prepared according to T.L.Fevig et al in J.Med.Chem 1987, 30, 156-165.

The above amine (1 mmol) and Cy 5.5 NHS ester (1 mmol) are dissolved in dimethylformamide (25) ml). N-methylmorpholine (300 μl) is added and the mixture stirred at ambient temperature for 4 days. The mixture is evaporated and the coupling product is isolated by flash chromoatography (silica, hexane and ethyl acetate). This product is dissolved in ethylacetate and treated with paratoluenesulphonic acid (25 mmol). The solvent is evaporated and the oily mixture is heated at 40 °C for 30 minutes. The product is isolated by flash chromatography (silica, hexane and ethyl acetate).

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Claims:

1. A contrast agent for optical imaging with affinity for an abnormally expressed biological target associated with endometrioses.

2. A contrast agent as claimed in claim 1 with molecular weight below 10000 Daltons.

3. A contrast agent as claimed in claim 1 or 2 of formula I

V-L-R, (I)

wherein V is one or more vector moieties having affinity for an abnormally expressed target in endometriosis, L is a linker moiety or a bond and R is one ore more reporter moieties detectable in in vivo optical imaging.

4. A contrast agent as claimed in any of claims 1 to 3 comprising a contrast agent substrate, wherein the target is an abnormally expressed enzyme, such that the contrast agent changes pharmacodynamic properties and/or pharmacokinetic properties upon a chemical modification from a contrast agent substrate to a contrast agent product upon a specific enzymatic transformation.

5. A contrast agent as claimed in any of claims 1 to 4 having affinity for any of the receptors selected from angiogenesis targets, adhesion molecules, estrogen receptors, progesterone receptors, Cathepsin H and Cathepsin S, aromatase, reductase, CD10, endoglin, haptoglobin and cyclin D2.

6. A contrast agent as claimed in claims 3 or 4 wherein V is selected from peptides, peptoid moieties, oligonucleotides, oligosaccharides and fat-related compounds.

7. A contrast agent as claimed in any of claims 3-6 wherein R is a dye that interacts with light in the wavelength region from the ultraviolet to the infrared part of the electromagnetic spectrum.

8. A pharmaceutical composition for optical imaging for diagnosis of endometriosis, for follow up of progress of endometriosis, or for follow up of treatment of endometriosis, comprising a contrast agent as defined in any of claims 1 to 7 together with at least one pharmaceutically acceptable carrier or excipient.

9. Use of a contrast agent as claimed in any of claims 1 to 7 for the manufacture of a diagnostic agent for use in a method of optical imaging of endometriosis involving administration of said diagnostic agent to an animate subject and generation of an image of at least part of said subject.

10. A method of generating an optical image of an animate subject involving administering a contrast agent to said subject and generating an optical image of at least a part of said subject to which said contrast agent has distributed, characterized in that as said contrast agent is used a contrast agent as defined in an any of claims 1 to 7.

11. Method as claimed in claim 10 for diagnosis of endometriosis, for follow up the progress of endometriosis development or for follow up treatment of endometriosis using a contrast agent as defined in any of claims 1 to 7.

12. Use of a contrast agent as defined in any of claims 1 to 7 for optical imaging of endometriosis.

Abstract

The invention provides contrast agents for optical imaging of endometriosis in patients. The contrast agent may be used in diagnosis of endometriosis, for follow up of progress in disease development, and for follow up of treatment of endometriosis. Further, the invention provides methods for optical imaging of endometriosis.

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